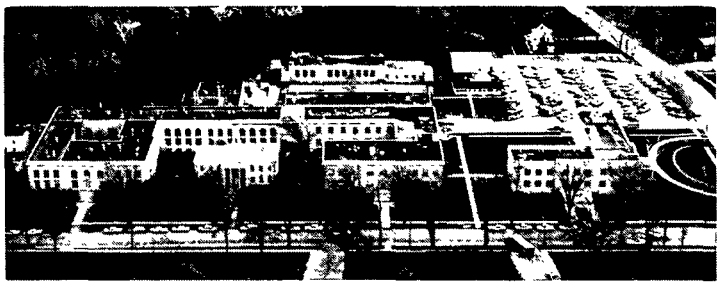

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GEL PERMEATION CHROMATOGRAPHIC ANALYSIS OF CELLULOSE AND WOOD PULP POLYSACCHARIDES

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Gel permeation chromatographic analysis of cellulose and wood pulp polysaccharides

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ABSTRACT

A method for gel permeation chromatographic analysis of the molecular weight distribution of wood pulp holocelluloses as the carbanilate derivatives has been applied to both red maple (Acer rubrum) and loblolly pine (Pinus taeda). Either chlorine-ethanolamine or acid-chlorite holocelluloses could be employed if the appropriate conditions for preparing the holocelluloses were employed. The holocelluloses were carbanilated with phenyl isocyanate in pyridine at 80°C. Higher temperatures resulted in some polysaccharide depolymerization.

Keywords: Gel permeation chromatography; molecular weight distribution; holocellulose; carbanilates; acer rubrum; pinus taeda; pulps; polysaccharides

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The major problem in determining the molecular weight distribution of wood polysaccharides is conversion of the polysaccharides to derivatives suitable for analysis. The two basic approaches to this problem are derivatization of the polysaccharides in the presence of the associated lignin or removal of the lignin, i.e., preparation of the holocellulose, before derivatization.

The only potentially useful method for derivatizing the polysaccharides without delignification is nitration (1-4). However, the stability of the nitrates, both dry and in solution, is limited, and there is risk of degradation during nitration (2,5-8). Also, nitrated xylans are notoriously insoluble (2,3) and would be excluded from the analysis. Finally, complete nitration is difficult to achieve, complicating the interpretation of analytical results (2,3,5,7). Thus, derivatization of the holocellulose was selected as the basis for the analytical procedure, even though some potential exists for depolymerization of the polysaccharides during preparation of the holocellulose (1,2). The chlorine-monoethanolamine holocellulose procedure (9) is preferred for quantitative isolation of the polysaccharides (2), but it is very tedious and time consuming. The acid-chlorite procedures (2) typically used for viscosity measurements are more suitable for routine analytical procedures because of their simplicity and the possibility of treating several samples simultaneously. Thus, both methods were studied.

The holocelluloses were derivatized with phenyl isocyanate to form the carbanilate (Fig. 1). Carbanilation has repeatedly been reported to provide complete substitution of polysaccharides without degradation (6-8,10) and has been used in gel permeation chromatographic (GPC)

analysis of cellulose (7,8) and bleached pulps containing up to 20% hemicellulose (6). Also, in contrast to the nitrates, xylan carbanilates are soluble in GPC solvents (11). A potential alternative to carbanilation, dissolution of the holocellulose in dimethylsulfoxide-paraformaldehyde (12), was not used because the resultant methylol derivatives are difficult to characterize (13).

[Fig. 1 here]

GPC analysis of pulp holocelluloses

Determination of the molecular weight or degree of polymerization (DP) distribution of a pulp holocellulose involves separation of the polysaccharides according to size, and evaluating both the size and relative proportions of the polymers. In GPC the derivatized polysaccharides are separated by eluting them through columns of porous material. The ability of the polysaccharides to penetrate the pores of the column packing is inversely proportional to their size (DP). Thus, the largest molecules elute first, the smallest molecules last. Interpretation of the elution curve depends upon calibration of the system, which is the correlation between the DP of the polysaccharides and their elution time or elution volume.

The utility of the GPC method, in its current state of development, is illustrated by analyses of carbanilated holocelluloses of fiberized red maple (14) (FRM) and a 65% yield oxygen-alkali pulp (OA-65) from FRM (Fig. 2). The chromatogram of FRM exhibits two maxima in addition to the internal reference. The high molecular weight maximum (lowest elution volume) is due to cellulose, while the low molecular weight maximum is indicative of the substantial hemicellulose content. The

lower hemicellulose content of the oxygen pulp is quite obvious. In addition, the overall DP values for the oxygen pulp are lower (larger elution volumes) than those for the FRM. Quantitatively, the pulp had a $\overline{DP}_w$ of 1230 while that for FRM was 2000.

[Fig. 2 here]

Polysaccharide carbanilation

Initially, cellulose and holocelluloses were carbanilated with phenyl isocyanate at reaction conditions reported as being nondegrading; pyridine solvent at 110°C or reflux (6-8), or N,N-dimethylformamide (DMF) solvent with triethylenediamine catalyst at 95-100°C (10). However, inconsistent DP analyses led us to suspect that degradation was occurring. To evaluate this possibility pyridine solutions of phenyl isocyanate and purified cellulose carbanilate, simulating derivatization conditions, were maintained at 110°C and reflux conditions. Periodically, the solutions were sampled for analysis of the carbanilate by GPC. The results for 110° and reflux conditions were essentially the same (Fig. 3). The $\overline{DP}_w$ of the cellulose carbanilate decreased steadily from ca. 2500 to ca. 650 over 3 days. Similar results were obtained in the DMF system. In addition, deliberately blanketing a reflux reaction with nitrogen to avoid potentially deleterious effects of oxygen did not alter the result (Fig. 3). However, at 80°C the carbanilate $\overline{DP}_w$ was essentially unchanged for at least two days. Thus, in subsequent carbanilations of holocelluloses a two-day reaction time and 80°C were used.

[Fig. 3 here]

To determine whether representative substitution of the polysaccharides was being effected with the milder, nondegrading carbanilation conditions, carbanilates of cellulose and several chlorine-ethanolamine

holocelluloses of red maple pulps prepared by the method were analyzed for their nitrogen content. The nitrogen analyses were in good agreement (± 1.6%) with theoretical values based on normalized carbohydrate analyses of the original pulps, thus indicating that substitution on the isolated polysaccharides was essentially complete and that the holocelluloses and their derivatives were representative of the polysaccharides in the initial pulp even though the yields were not quantitative.

Holocellulose preparation

The number of chlorinations required to prepare a holocellulose by the chlorine-monoethanolamine procedure is quite arbitrary. The end point is typically defined as attainment of a white sample or no further color change on successive chlorinations (2,9). We were curious whether degradation could occur with additional inadvertent chlorinations. In preparing a holocellulose from fiberized loblolly pine, 7 chlorination-extraction sequences were judged to be sufficient. At that point the $\overline{DP}_w$ of the holocellulose was 2120. After 9 chlorination-extraction sequences the $\overline{DP}_w$ was 2100. Thus, two additional chlorinations past the arbitrary end point did not affect the DP of the holocellulose.

While the chlorine-monoethanolamine holocellulose procedure appears to be the preferred method for quantitative isolation of the polysaccharides (2), it is tedious and time-consuming. Therefore, the more convenient acid-chlorite procedures (2) which are frequently used to prepare holocelluloses for viscosity measurements were also evaluated. The DP analyses for holocelluloses prepared from red maple pulps by both the acid-chlorite and chlorine-monoethanolamine procedures are presented in Table I. When the lignin content of the sample was very high, as in the

fiberized chip and the 90% yield pulp, the DP values for the acid-chlorite and chlorine-monoethanolamine holocelluloses were drastically different, with the acid-chlorite holocelluloses yielding much lower DP values. However, with samples of lower lignin content the DP values of the two types of holocellulose were comparable. The result cannot be reasonably attributed to excessive degradation in the acid-chlorite system since the samples were treated similarly, and, if this were the case, it might be anticipated that carbohydrate degradation would have been most severe in the samples containing the least amount of lignin. Rather, the differences in the DP values can be attributed to inaccessibility of the carbohydrates to the derivatization reagents in the acid-chlorite holocelluloses due to incomplete delignification. On this basis, it was anticipated that the problem with the acid-chlorite procedure might be eliminated by use of more reagent (lower consistency), higher temperatures, or multiple treatments of samples with high lignin contents.

[Table I here]

The effects of temperature and lower consistency on the apparent DP of acid chlorite holocelluloses of fiberized loblolly pine are illustrated in Table II. Increasing the temperature of a one-day, 5%-consistency acid-chlorite treatment of the pine increased the apparent DP values of the holocellulose, until at 40°C the $\overline{DP}_w$ was essentially the same as that obtained by the chlorine-monoethanolamine procedure. Similarly, the effect of multiple acid-chlorite treatments at 25°C is shown in Table II. After two treatments the $\overline{DP}_n$ and $\overline{DP}_w$ values for the holocelluloses were essentially the same, and equal to values obtained by the chlorine-monoethanolamine method. Thus, the problems initially encountered with the

acid-chlorite holocellulose procedure could be circumvented by judicious adjustment of the reaction conditions.

[Table II here]

Experimental

Chlorine-monoethanolamine holocelluloses

The procedure of Holmes and Kurth (2,9) was used except that never-dried wood or pulp samples were used, and 0.01M $CaCl_2$ solution was substituted for aqueous washes. The holocellulose was slurried with water, and the mixture was frozen and freeze-dried. Freeze-drying gave a material which was more easily dispersed during derivatization.

Acid-chlorite holocelluloses

Water (99 mL), sodium chlorite (5 g) and glacial acetic acid (1 g) were mixed together. The chlorite solution (20 mL) and a sample (ca. 2 or 1 g o.d., depending on the desired consistency) of never-dried pulp were placed in a tightly-stoppered test tube in a light-protected water bath at the desired temperature for 24 hr. For repetitive treatments, the chlorite solution was decanted, fresh chlorite solution was added, and the 24-hr treatment was repeated. The holocellulose was washed with 5 liters of distilled water, slurried with 50 mL of distilled water, frozen, and freeze-dried.

Holocellulose carbanilation

The holocellulose was dried at least overnight in vacuo over phosphorus pentoxide. Anhydrous pyridine (15) (100 mL), holocellulose (0.1 g), and phenyl isocyanate (7.2 mL) were sealed in a 4-oz bottle with a Teflon-lined cap. The reaction bottles were sealed in steel laboratory

digesters of slightly larger diameter and rotated at ca. 3 rpm in an oil bath at 80°C for 2 days. The mixture was allowed to cool slightly and methanol (4 mL) was added to react with the excess phenyl isocyanate.

The mixture was centrifuged, and the supernatant was mixed with an equal volume of dioxane. The solution was filtered through dry, washed sand over two glass filter pads, diluted with 10-20% dioxane, and poured in a fine stream into a stirred solution of methanol (800 mL) and acetic acid (5 mL). The precipitated polymer was washed with water (800 mL) containing acetic acid (5 mL), washed with water (800 mL), and freeze-dried.

GPC analyses

Tetrahydrofuran solutions of carbanilated cellulose or holocellulose with an internal reference, methyl N-phenylcarbamate, were analyzed on Styragel columns (Waters Associates) having permeability ranges of 10^6, 10^5, 10^4, and 10^3 A. Freshly purified tetrahydrofuran (15) was used as the elution solvent at a flow rate of 2.0 mL min^{-1}. The spectrophotometric detector was operated at 235 nm.

The GPC columns were calibrated by the universal technique (7,16,17) using eleven "monodisperse" polystyrene standards with molecular weights from 2.1×10^3 to 3.6×10^6. Mark-Houwink constants, K and α, used in the calibration were those reported by Valtasaari and Saarela (7); 1.8×10^{-2} mL g^{-1} and 0.74, respectively, for polystyrene and 2.01×10^{-3} mL g^{-1} and 0.92, respectively, for cellulose tricarbanilate. El Ashmawy, et al. (6) reported similar constants for the carbanilate of an 80% α-cellulose sulfite pulp; 2.51×10^{-3} mL g^{-1} and 0.89, respectively. From the cali-

bration, a curve relating the molecular weight of the polysaccharide carbanilates (M_i) to their elution volume (V_i), relative to the elution volume (V_s) of the reference compound, was obtained (Fig. 4).

[Fig. 4 here]

In analyses of chromatograms the base line was defined as the line between a point on the elution curve at a $V_s - V_i$ value of 15 and the start of polymer elution (Fig. 2). The ordinate heights (h_i) were determined at $V_s - V_i$ intervals of 2 mL. This provided at least 20 data points over the elution curve. The number-average ($\overline{M}_n$), weight-average ($\overline{M}_w$), and viscosity-average ($\overline{M}_v$) molecular weights could be calculated according to Equations (1), (2), and (3), respectively. Apparent average degrees of polymerization ($\overline{DP}_n$, $\overline{DP}_w$, and $\overline{DP}_v$) were calculated by dividing the respective molecular weight values by 519, the monomer equivalent weight of cellulose tricarbanilate.

$$\overline{M}_n = \Sigma h_i / \Sigma (h_i / M_i) \quad (1)$$

$$\overline{M}_w = \Sigma h_i M_i / \Sigma h_i \quad (2)$$

$$\overline{M}_v = [\Sigma h_i M_i^{\alpha} / \Sigma h_i]^{1/\alpha} \quad (3)$$

Literature cited

1. Browning, B. L. In Browning's "The Chemistry of Wood." Wiley-Interscience, New York, 1963. Chapter 3.

2. Browning, B. L. "Methods of Wood Chemistry," Vol. II. Wiley-Interscience, New York, 1967. Chapter 19.

3. Timell, T. E., Methods Carbohyd. Chem. 5: 100 (1965).

4. Spencer, F. S. and MacLachlan, G. A., Plant Physiol. 49: 58 (1972).

5. Green, J. W., Methods Carbohyd. Chem. 3: 218, 222 (1963).

6. El Ashmawy, A. E., Danhelka, J., and Kössler, I., Svensk Papperstidn. 77: 603 (1974).

7. Valtasaari, L. and Saarela, K., Paperi Puu 57: 5 (1975).

8. Danhelka, J., Kossler, I., and Bohackova, V., J. Polymer Sci. (Polymer Chem.) 14: 287 (1976).

9. Holmes, G. W. and Kurth, E. F., Tappi 42: 837 (1959).

10. Hall, D. M. and Horne, J. R., J. Appl. Polymer Sci. 17: 3727 (1973).

11. Driss, M. and Pla, F., ATIP Rev. 26: 467 (1972).

12. Johnson, D. C., Nicholson, M. D., and Haigh, F. C., Appl. Polymer Symp. 28: 931 (1976).

13. Baker, T. J., Schroeder, L. R., and Johnson, D. C., Carbohyd. Res. 67: C4 (1978).

14. Nicholls, G. A., Jamieson, R. G., and Van Drunen, V. J., Tappi 58(5): 105 (1975).

15. Perrin, D. D., Armarego, W. L. F., and Perrin, D. R., "Purification of Laboratory Chemicals," Pergamon Press, London, 1966.

16. Hamielec, A. E. and Ouano, A. C., J. Liquid Chromatog. 1(1): 111 (1978) and references cited therein.

17. Billingham, N. C., In Simpson's "Practical High Performance Liquid Chromatography," Heyden and Son, London, 1976. Chapter 10.

I. Effect of holocellulose preparation on the observed degree of polymerization of red maple pulps

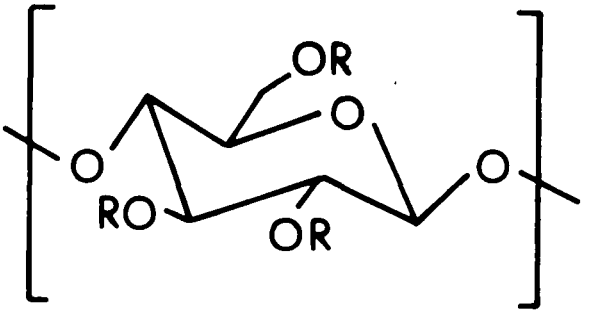
	Neutral carbohydrate, %	Holocellulose method[a]	$\overline{DP}_w$
Fiberized red maple	65	C	2000
		AC	1340
90% yield pulp	66	C	1930
		AC	1260
72% yield pulp	74	C	1490
		AC	1560
65% yield pulp	82	C	1430
		AC	1460

[a]C, chlorine-ethanolamine procedure; AC, acid-chlorite procedure, one-day 25°C, 10% consistency.

II. Effect of temperature and successive treatments on the observed degree of polymerization of acid-chlorite holocelluloses from fiberized loblolly pine

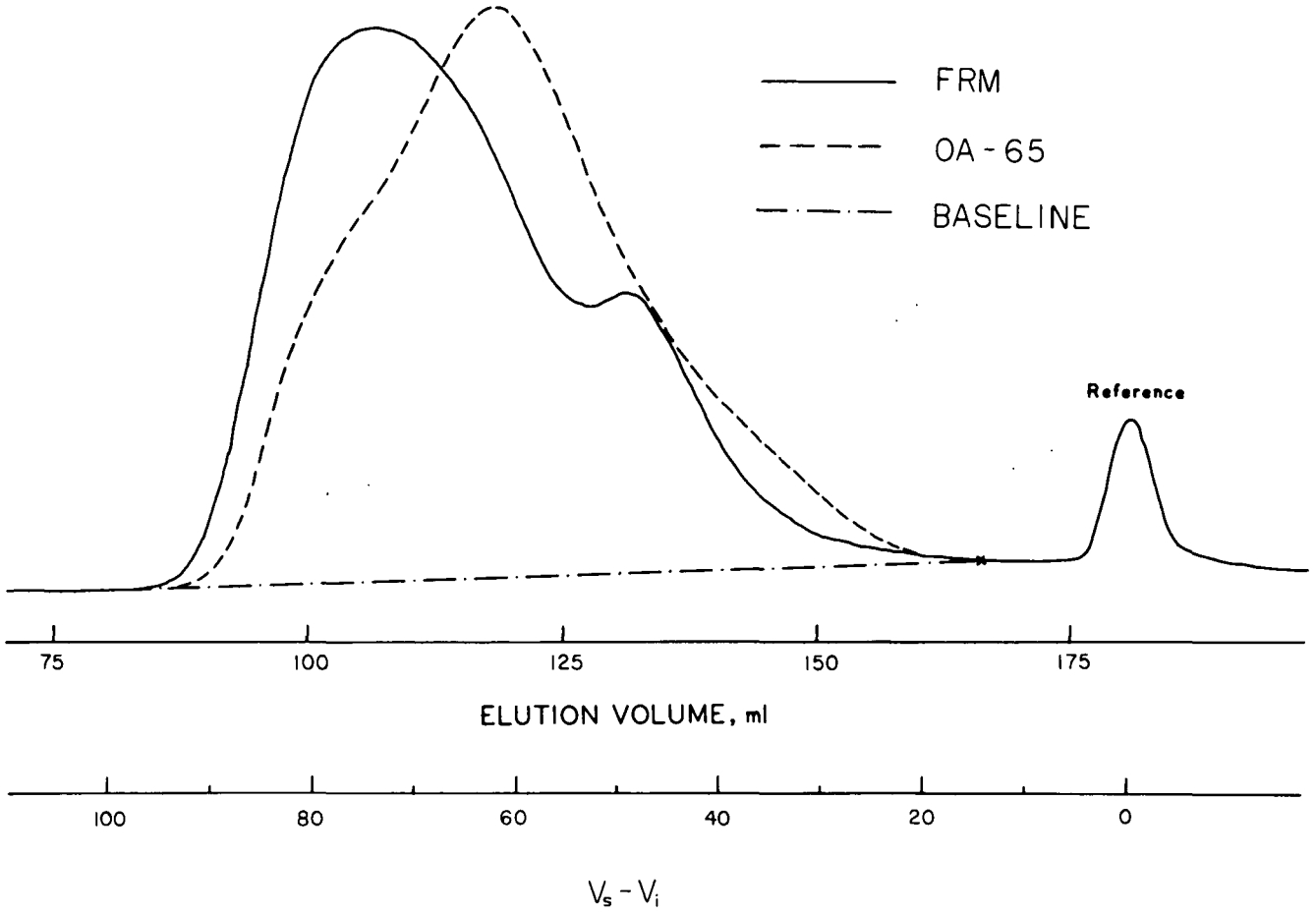
Temperature, °C	Treatments[a]	$\overline{DP}_n$	$\overline{DP}_w$
25	1	430	1710
30	1	460	1840
35	1	480	1920
40	1	540	2150
45	1	530	2130
25	2	580	2140
25	3	590	2120
25	4	570	2250
25	5	560	2160

[a]One day, 5% consistency.

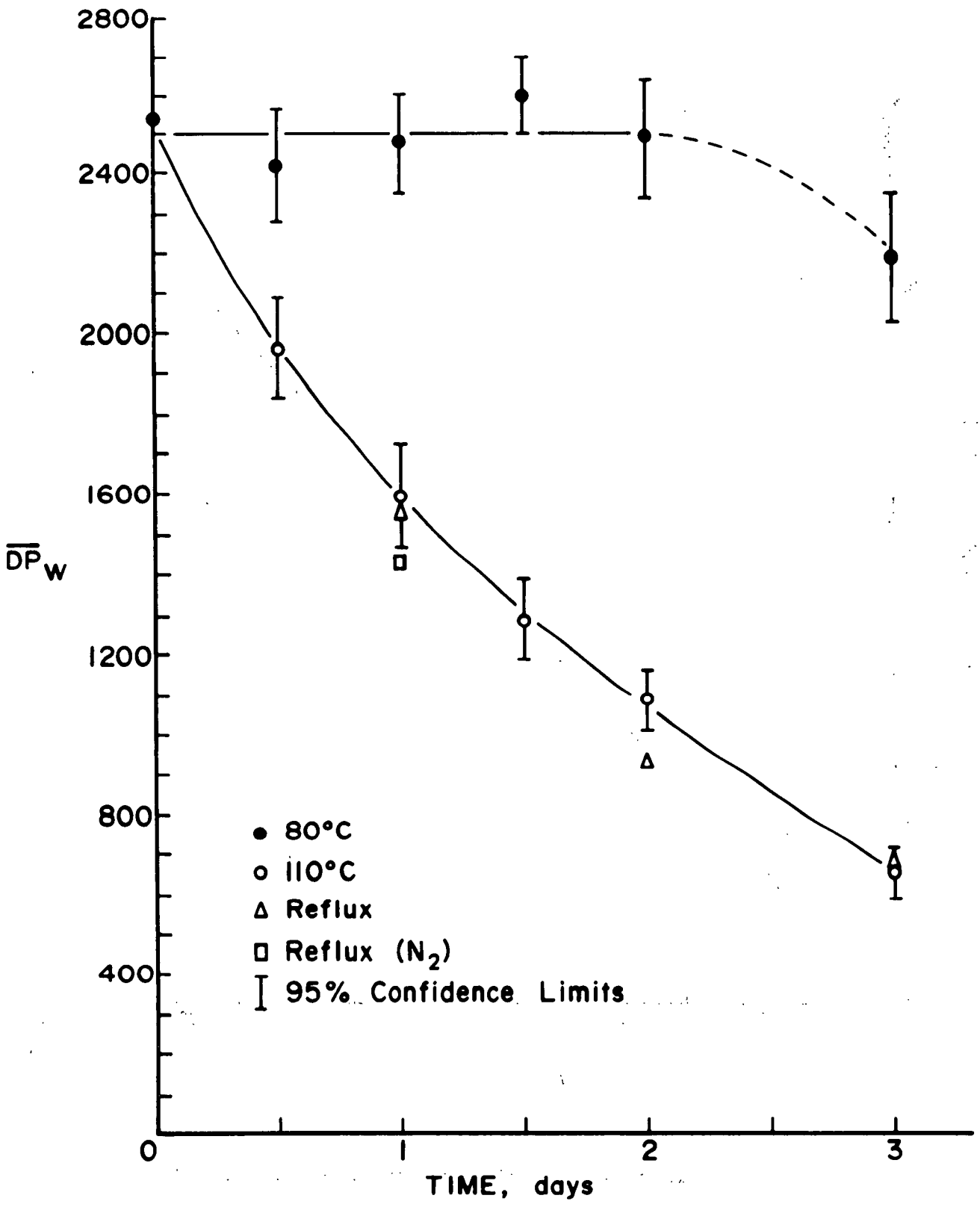


$$R = -\overset{\overset{O}{\|}}{C}-\overset{\overset{H}{|}}{N}-C_6H_5$$

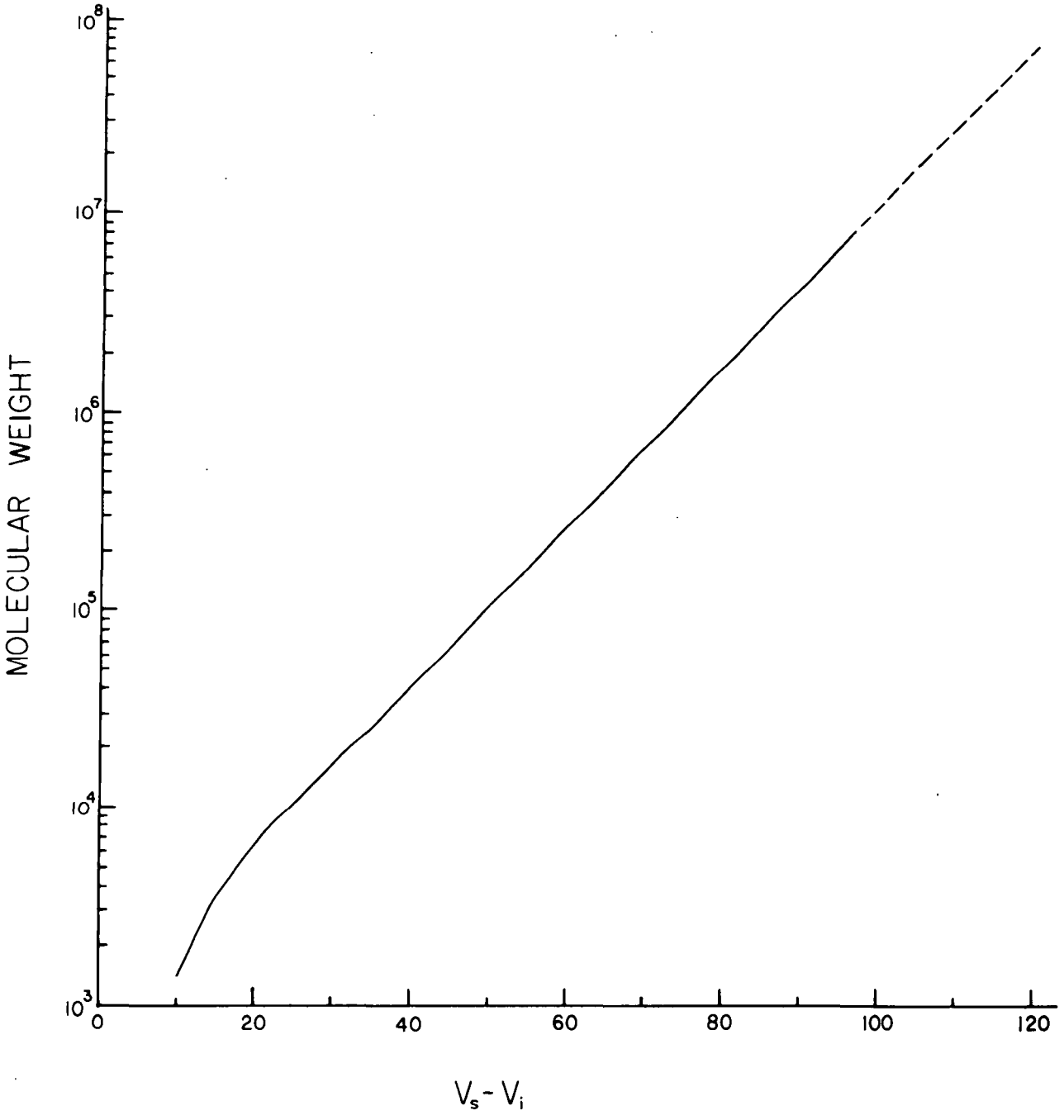
1. Monomer unit of cellulose tricarbanilate.



2. GPC analyses of polysaccharides in fiberized red maple (FRM) and a 65%-yield oxygen-alkali pulp from FRM (OA-65).



3. Stability of cellulose tricarbanilate to various carbanilation conditions in pyridine.



4. Cellulose tricarbanilate molecular weight versus relative elution volume.